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## Pathophysiology

# Sodium–glucose cotransporter 2 inhibition: cardioprotection by treating diabetes—a translational viewpoint explaining its potential salutary effects

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Diabetes is a growing epidemic worldwide characterized by an elevated concentration of blood glucose, associated with a high incidence of cardiovascular disease and mortality. Although in general reduction of hyperglycaemia is considered a therapeutic goal, hypoglycaemic therapies do not necessarily reduce cardiovascular mortality and may even aggravate cardiovascular risk factors, such as body weight. A new class of antidiabetic drugs acts by inhibition of the sodium–glucose cotransporter 2 (SGLT2), which (partially) prevents reabsorption of glucose from the renal filtrate. The induction of glucose excretion via the urine (glycosuria) was turned into an effective strategy to reduce blood glucose. Ancillary advantages are the caloric and volumetric loss and thereby the reduction of body weight and blood pressure. Additionally, SGLT2 inhibition has been suggested to exert direct cardioprotective effects by the reduction of cardiac fibrosis, inflammation, and oxidative stress. This article summarizes the functional consequences of SGLT2 inhibition on the diabetic and hyperglycaemic organism. We especially focused on the effects on the kidney and the cardiovascular system as described in experimental studies. The interesting observations in experimental studies may extend to clinical medicine, as a recent trial reported a decrease in heart failure outcomes in patients at high cardiovascular risk. In conclusion, SGLT2 inhibition represents a novel treatment, which might be a promising target not only to (further) reduce blood glucose but also to target other cardiovascular risk factors. More research and long-term follow-ups will reveal the specific influence of SGLT2 inhibition on the circulatory system and cardiovascular outcomes.

**Keywords** SGLT2 inhibition • Diabetes mellitus • Hyperglycaemia • Antidiabetic agent • Heart failure • CV event

## Introduction

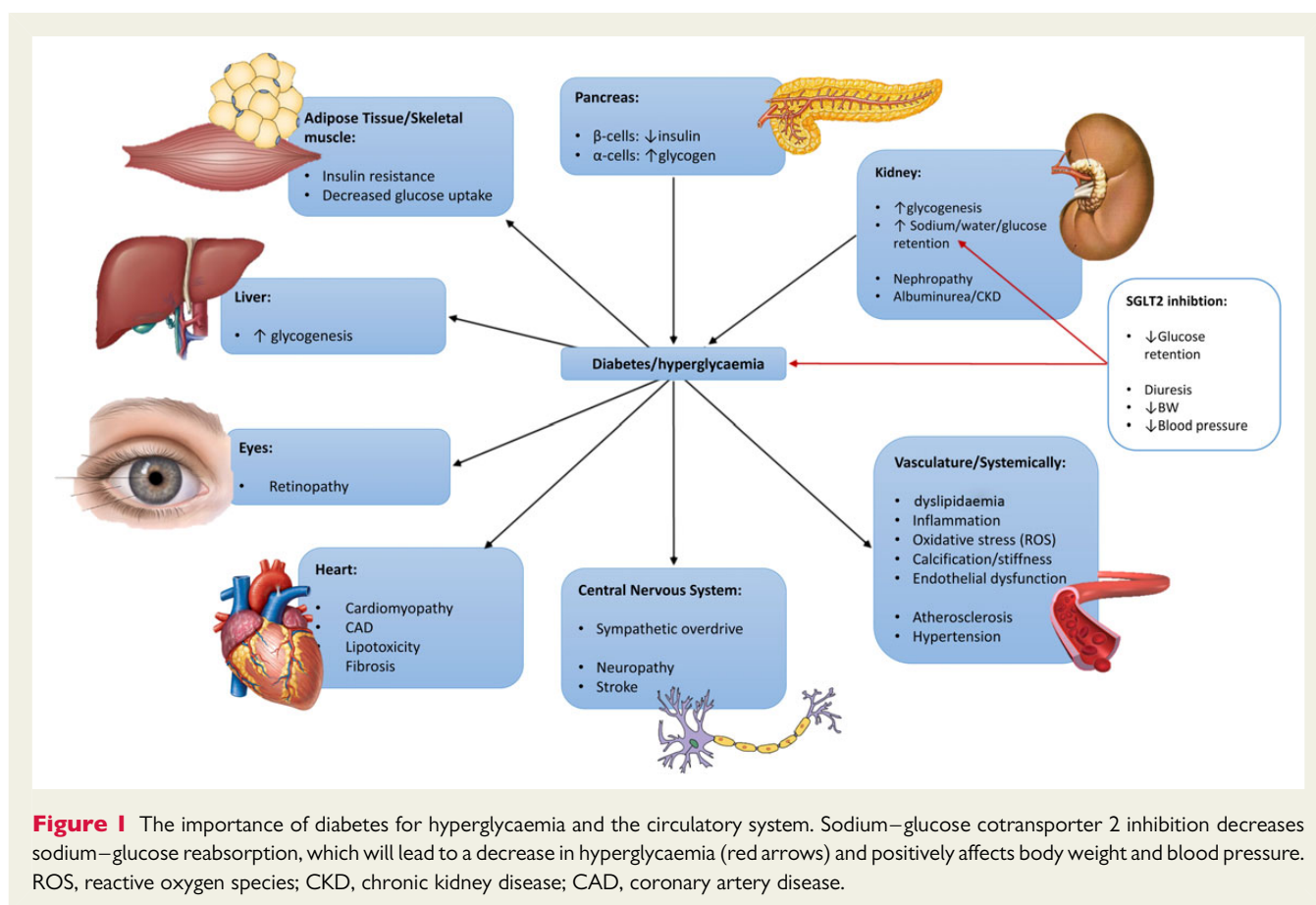
Diabetes is a growing epidemic due to population growth, ageing, urbanization, and westernization.<sup>1,2</sup> Hereby, the burden of diabetes translates into substantial social and economic problems.<sup>1</sup> Cardiovascular disease (CVD) is very common in diabetic subjects and is the most frequent cause of death in these patients.<sup>3–5</sup> Diabetic hyperglycaemia causes a number of microvascular and macrovascular complications (Figure 1).<sup>4,6,7</sup> The alleviation of hyperglycaemia, often by oral antidiabetic drugs, leads to improvement of insulin sensitivity,  $\beta$ -cell function, and reduction of microvascular complications such as retinopathy, neuropathy, and nephropathy.<sup>4,6,8–11</sup> Several oral antidiabetic drugs are in use, including metformin, sulphonyl urea derivatives, dipeptidyl peptidase-4 (DPP-4)

inhibitors, and thiazolidinediones. However, current therapies targeted to reduce hyperglycaemia tend to lose their effectiveness over time and are associated with negative side effects, including weight gain and hypoglycaemia.<sup>1,12,13</sup> Furthermore, standard anti-hyperglycaemic treatments do not necessarily confer full protection against CVDs, such as coronary artery disease (CAD), stroke, and peripheral vascular disease as shown in several large, long-term outcome studies,<sup>11,13–17</sup> partially explained by the risk at hypoglycaemic events. Furthermore, in order to effectively reduce cardiovascular (CV) events in diabetic patients, co-morbidities such as obesity, hypertension, and hypercholesterolaemia must be co-targeted, in conjunction to the maintenance of glycaemic control.<sup>4,18</sup>

Recently, a new class of drugs, the sodium–glucose cotransporter 2 (SGLT2) inhibitors, was introduced, which reduce blood

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glucose by (partially) blocking its reabsorption in the kidneys leading to glucose excretion via the urine.<sup>12</sup> Glucose reabsorption is mainly orchestrated by the SGLT2.<sup>12,19,20</sup> A number of well-tolerated SGLT2 inhibitors are currently being tested in clinical trials as glucose-lowering medication.<sup>2,19,21–23</sup> SGLT2 inhibition might have neutral or positive effects on CV risk factors by volume depletion and caloric loss (Figure 1).<sup>22</sup> Additionally, because the mode of action does not influence insulin secretion or sensitivity, a larger number of diabetic patients can be targeted, possibly also patients with type 1 diabetes mellitus (T1DM).<sup>24</sup>

Although short-term outcomes on safety and efficacy of SGLT2 inhibition to target hyperglycaemia are promising, the long-term outcomes on CVD are presently unclear.<sup>25</sup> This review will summarize the physiology of SGLT2 inhibition, and clinical and pre-clinical data are collected in an effort to clarify the impact of SGLT2 inhibition on CV risk factors and outcomes.

## Renal glucose reabsorption by sodium–glucose cotransporter in diabetes

The kidneys are vital organs in regulating the glucose balance by gluconeogenesis, filtration, and reabsorption.<sup>12,26,27</sup> When blood glucose is at normal levels, the kidneys filter ~180 g of glucose per day.<sup>27</sup> Virtually all glucose filtered in the glomerulus is

reabsorbed and as a result (almost) no glucose is excreted in the urine (<0.5 g/day).<sup>26,27</sup> Glucose transport is bound to a maximum threshold ( $Tm_G$ ).<sup>28,29</sup> Glycosuria will occur whenever this maximum is exceeded.<sup>22,29</sup>

Renal glucose control is generally disturbed in diabetes, which further exacerbates hyperglycaemic conditions.<sup>26</sup> Gluconeogenesis in the diabetic kidney can be increased up to 300%, while diabetic glomerular filtration rate (GFR) may increase or exceed up to 120 mL/min/1.73 m<sup>2</sup>, leading to reabsorption of glucose of ~300 g/day.<sup>26–28</sup> Additionally,  $Tm_G$  is increased in both T1DM and T2DM.<sup>28,29</sup> By targeting the function of SGLT in the kidneys, theoretically  $Tm_G$  could decrease as well as the amount of glucose reabsorption, which may benefit the severity of disease.

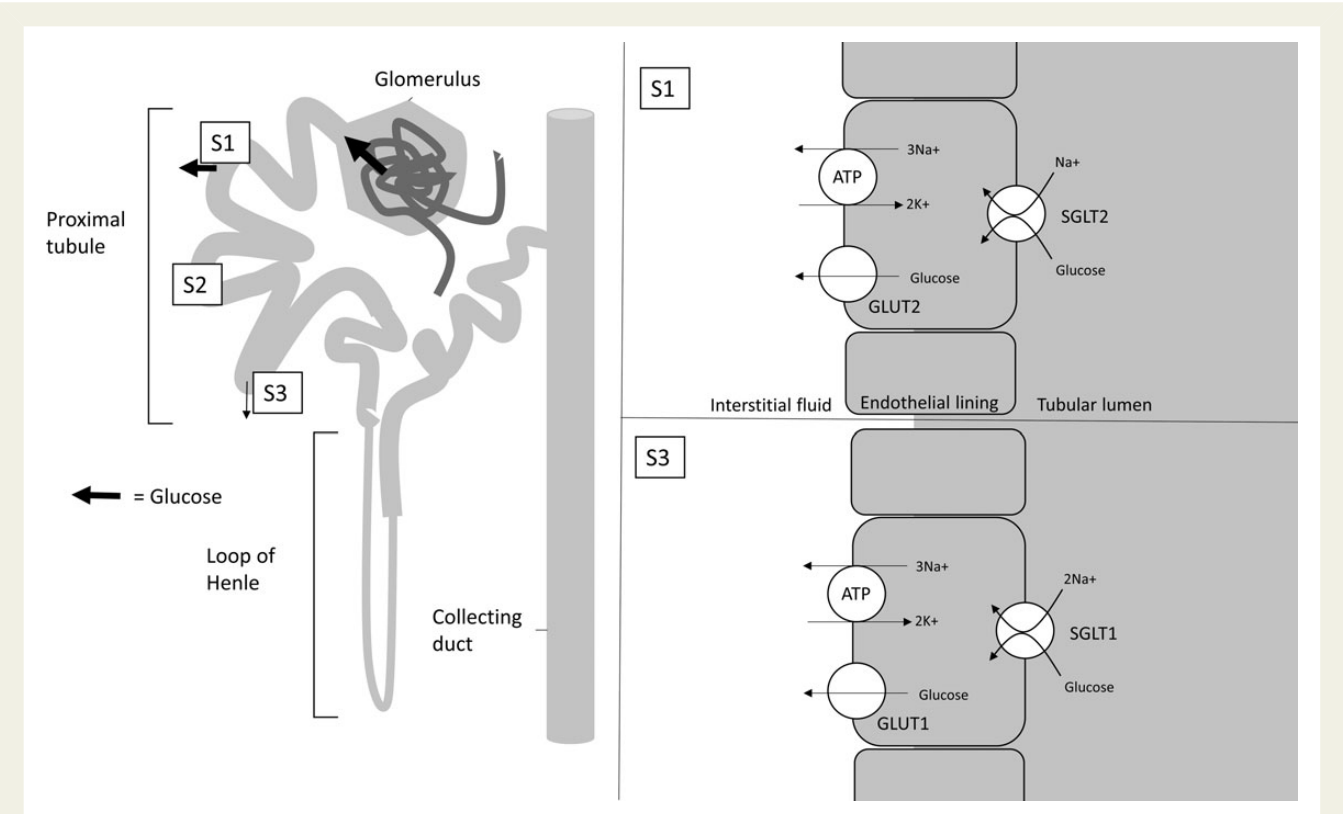
## Distribution and function of sodium–glucose cotransporters

SGLTs utilize the electrical gradient of sodium to transport glucose over cellular membranes.<sup>30</sup> They habitually collaborate with glucose transporters (GLUTs) to facilitate glucose transport into the interstitial fluid after reabsorption (by SGLT) from the lumen.<sup>12</sup> Most SGLTs (1, 2, and 4–6) are transcribed from the *SLC5* gene. SGLT1 and SGLT2 are the predominant transporters with variable expression in several tissues (Table 1).<sup>20</sup>

Both SGLT1 and SGLT2 are expressed in the kidney (Figure 2).<sup>32,33</sup> SGLT2 is a 'low-affinity high-capacity' transporter, due to a very condensed expression in the S1 segment of the proximal tubule

**Table 1** Tissue distribution and substrate for sodium–glucose cotransporters 1 and 2<sup>31</sup>

Transport	Substrate	Distribution
SGLT1 (SLC5A1)	Glucose, galactose, mannose	Intestine, trachea, kidney, heart, brain, testis and prostate
SGLT2 (SLC5A2)	Glucose (and galactose, not transported)	Kidney, brain, liver, thyroid, muscle, and heart



**Figure 2** Regulation of glucose transport by the kidney. The left panel depicts glucose transport (black arrows). Most glucose is reabsorbed in the S1 segment by sodium–glucose cotransporter 2. The top right picture shows how the sodium–glucose cotransporter 2 transporter binds one glucose molecule and one sodium ion to transport glucose from the tubular lumen. Here, glucose transporter 2 pumps glucose into the interstitial fluid. Sodium–glucose cotransporter 1 binds one glucose and two sodium molecules where it co-operates with glucose transporter 1. Sodium is actively pumped out of the cell by the Na<sup>+</sup>/K<sup>+</sup> pump. SGLT, sodium–glucose cotransporter; GLUT, glucose transporter.

of the kidney.<sup>33,34</sup> SGLT1 is a ‘high-affinity low-capacity’ transporter expressed in the more distal segment (S3).<sup>33,34</sup> The SGLT2 transporter is considered responsible for 90% of all glucose reabsorption, confirmed by studies using kidney-specific depletion of the SGLT2 gene.<sup>22,32,34</sup> SGLT1 primarily exerts a role in glucose absorption of the small intestine.<sup>31,35</sup> SGLT1 may also play a role in glucose reabsorption in the late proximal tubule in the event that the function of SGLT2 is comprised.<sup>12,20,36</sup> However, no compensatory role of SGLT1 has been described during SGLT2 inhibition.<sup>37</sup> Nevertheless, the maximum degree of inhibition of glucose reabsorption does not exceed 30–50%.<sup>38</sup> Specific SGLT2 inhibition is therefore anticipated to result in substantial glycosuria, leading to a decrease in plasma glucose, with a low risk for hypoglycaemic events.<sup>39</sup>

### Renal glucose transport in hyperglycaemia

SGLT2 works at 50% capacity and becomes saturated when the glucose concentration exceeds >35 mM.<sup>20</sup> However, SGLT expression levels are increased in diabetes. A study in Akita (T1DM) mice and STZ-induced diabetic rats has shown that SGLT2 expression in the renal membrane increases in DM, and SGLT1 expression was decreased in comparison with their healthy littermates.<sup>40</sup> Furthermore, others studies reported an increase in SGLT2 synthesis in hyperglycaemic conditions and increased SGLT2 and GLUT2 expression on isolated proximal tubular cells from fresh urine in humans.<sup>41,42</sup> These findings may correspond to the increased level of Tm<sub>G</sub> and glucose reabsorption in diabetic patients.<sup>27,42</sup>

## Sodium–glucose cotransporter 2 deficiency

Transgenic mouse models have been created by disruption of the *SLC5A* gene.<sup>43,44</sup> It has been shown that the sweet pee model of Lee *et al.*, when diabetes is induced, has improved glycaemic control, although the mortality rate was increased in these groups, possibly due to kidney failure.<sup>43</sup> Experiments by Jurczak *et al.*, who crossed SGLT<sup>−/−</sup> with *ob/ob* mice, showed that SGLT deficiency was protective against the development of T2DM.<sup>44</sup> These results indicate that SGLT2 depletion leads to improved glycaemic control during hyperglycaemic conditions.

Human familial renal glycosuria is a rare, hereditary condition which may be caused by mutations in the *SCL5A2* gene, leading to different expression levels of SGLT2.<sup>45–47</sup> Patients with such mutations are characterized by excretion of glucose in the urine varying from 1 to 170 g/day, yet accompanied with normal plasma glucose concentrations.<sup>2,31,45</sup> Patients with full depletion of SGLT2, however, are diagnosed with massive glycosuria, sometimes combined with natriuresis and polyuria.<sup>46–48</sup> In these patients, no clinical or renal complications have been reported, which may prompt the consideration that glycosuria is a benign condition, and may be suitable as a treatment option for diabetic patients.<sup>31,45–49</sup> However, the number of patients studied was very low, while it cannot be excluded that other genes compensate in inherited conditions.

## Glycaemic effects of sodium–glucose cotransporter 2 inhibition

Phlorizin is a naturally occurring SGLT inhibitor, which is present in fruit trees. It has been evaluated for its glucose-lowering effects, and pre-clinical research showed promising data.<sup>8,40,50</sup> However, phlorizin turned out to be non-selective, which rendered it clinically not useful due to severe gastrointestinal side effects, such as osmotic diarrhoea.<sup>12,43</sup> An ideal target for reducing hyperglycaemia by inducing glycosuria should be selective, safe, and well tolerated.<sup>12,23</sup>

Later, specific SGLT2 inhibitors were developed and currently multiple SGLT2 inhibitors are under investigation in clinical trials. Examples are ipragliflozin,<sup>51,52</sup> empagliflozin,<sup>53</sup> remigliflozin,<sup>54</sup> and tofogliflozin.<sup>55</sup> Moreover, some SGLT2 inhibitors are already registered treatments for T2DM, such as empagliflozin, canagliflozin (USA),<sup>21,22,53,56</sup> and dapagliflozin (Europe).<sup>21–23,53,57,58</sup> These drugs were all tested and showed to have high affinity for SGLT2, low affinity for SGLT1, good bioavailability, and slow physical breakdown, allowing a (once) daily oral dose administration.<sup>53</sup>

Most clinical research has been performed on patients with T2DM (Table 2). Here, it has been reported that chronic SGLT2 inhibition for 24 weeks lowers the percentage of plasma glycated haemoglobin (HbA1c) levels by 0.5–2.5% depending on the dose, drug, and the baseline HbA1c levels.<sup>2,19,53,76–80</sup> For example, in patients with late-stage diabetes and thereby higher HbA1c (diagnosed for over 11–19 years), HbA1c decreased to an identical level, but twice as fast compared with other glucose-lowering drugs.<sup>81</sup>

Animal studies with SGLT2 inhibition have shown that fasting plasma glucose (FPG) was reduced up to 50% in hyperglycaemic or diabetic conditions, while there was no change in normoglycaemic conditions,<sup>36,54,82</sup> suggestive for a low risk of hypoglycaemic

events. Patient studies have shown a decrease in FPG from −1.0 to −3.3 mmol/L after 12 weeks treatment with either dapagliflozin, empagliflozin, or canagliflozin, which remained constant up to 72 weeks.<sup>59,60,63,71,73,78,83,84</sup> In comparison, patients with inadequate control of hyperglycaemia showed less of a decline, or even an increase in FPG when treated with a placebo next to their normal treatment.<sup>59,60,71,84</sup> These results indicated a quick decline in plasma glucose, which plateaus to a steady decrease after a longer period of time. Furthermore, SGLT2 inhibition appears to be a valuable add-on therapy in inadequately controlled patients to further reduce hyperglycaemia and to improve long-term stability of target values.<sup>61,63,83,85–89</sup> Additionally, increasing doses of insulin to remain a euglycaemic state were no longer required during SGLT2 inhibition, and therefore may improve insulin resistance and prevent negative side effects of insulin, such as increases in body weight.<sup>90</sup>

Other beneficial findings on SGLT2 inhibition are improvement of pancreatic  $\beta$ -cell function concerning glucose sensitivity,<sup>91</sup> cell mass and overall function,<sup>91–95</sup> and improvement in insulin sensitivity and associated peripheral glucose uptake.<sup>91,93,96,97</sup> SGLT2 inhibition also appears to directly affect pancreatic  $\alpha$ -cells, leading to an increase of glucagon and subsequent (hepatic) gluconeogenesis.<sup>91,96,98</sup>

## Non-glycaemic effects of sodium–glucose cotransporter 2 inhibition

Classical CV risk factors include age, smoking, hyperglycaemia, hypertension, dyslipidaemia, obesity (especially visceral) with sub-clinical inflammation, and endothelial dysfunction—conditions which are often interrelated (Figure 1).<sup>4,99,100</sup> Intensive glycaemic control with current therapies may alter several risk factors such as blood pressure and dyslipidaemia but negatively influence other risk factors such as body weight. Furthermore, the CV mortality and all-cause mortality are generally not decreased, indicating glycaemic control alone is not enough to tackle the problem of CV risk in diabetic patients.<sup>4,101,102</sup> As mentioned above, trials have suggested that SGLT2 inhibitors are able to lower HbA1c and fasting blood glucose with low risk of hypoglycaemia or serious events. Here, the impact on CV risk factors is described.

### Body weight

Body weight is a central factor in diabetes: 85% of all T2DM patients are overweight, and body weight increases with age, also in T1DM patients.<sup>3,7,99,103–105</sup> Obesity and metabolic syndrome are established independent predictors for CVD.<sup>100</sup> Risk of having a stroke or CAD doubles when diabetic patients are also overweight.<sup>3</sup>

SGLT2 inhibition may lead to a decrease in body weight by depletion of plasma volume, or by caloric loss of glucose via urine. Theoretically, SGLT2 inhibition by 20–25% could lead to loss of 60–80 g of glucose per day, which would equal 260–320 kcal.<sup>12,22,39</sup> Pre-clinical research has shown that SGLT2 inhibition is associated with a decrease (or sometimes no change) in body weight, which was often paired with an increase in caloric intake.<sup>55,82,95,106</sup> This could indicate that the increased caloric intake is compensated by increased energy expenditure in response to SGLT2 inhibitors in animal models. Furthermore, a reduction of

**Table 2** Selection of clinical trials with sodium–glucose cotransporter 2 inhibition as a treatment for diabetes

Compound	Comparator	n	Duration	Primary endpoint	References
T2DM					
Dapagliflozin	Placebo	546	24 weeks	Change from baseline in HbA1c	59
	Low dose–high dose				
Dapagliflozin	Placebo	485	24 weeks		60
	Low dose–high dose				
Dapagliflozin	Glipizide	814	52 weeks		61
Dapagliflozin	Placebo		24 weeks	HbA1c percentage from baseline	62
	Low dose–high dose				
	Glimepiride				
Dapagliflozin	Placebo	1236	24 weeks	HbA1c percentage from baseline	63
	Low dose–high dose				
Canagliflozin	Placebo	451	12 weeks		64
	Low dose–high dose				
	Once daily–twice daily				
Empagliflozin	Placebo	899	24 weeks	Change from baseline in HbA1c	65
	Low concentration				
	High concentration				
	Sitagliptin control				
Empagliflozin	Placebo	666	24 weeks	Change in HbA1c from baseline	66
	Low concentration				
	High concentration				
Empagliflozin	Placebo		12 weeks (FU 72 weeks)	Change in vital signs and clinical laboratory parameters from baseline	67
	Low dose–high dose				
Empagliflozin	Placebo	637	24 weeks	Change in HbA1c from baseline	68
	Low concentration				
	High concentration				
Empagliflozin	Placebo	498	24 weeks	Change in HbA1c from baseline	69
	Low concentration				
	High concentration				
Dapagliflozin	Placebo	140	102 weeks	Mean change in HbA1c from baseline	70
	Low dose–high dose				
	Insulin				
Canagliflozin	Placebo	342	26 weeks, (FU 52 weeks)	Mean change in HbA1c from baseline	71
	Low dose–high dose				
	Sitagliptin				
Empagliflozin	Placebo	825	12 weeks	Change in HbA1c and change in blood pressure (secondary)	72
	Low concentration				
	High concentration				
Canagliflozin	Placebo	676	18 weeks	Reduction in HbA1c	73
	Low dose–high dose				
Empagliflozin	Placebo	7020	3 years	Death from cardiovascular causes, nonfatal myocardial infarction or nonfatal stroke	74
	Low dose–high dose				
Empagliflozin	Placebo	267	52 weeks	Change in HbA1c from baseline	Recruiting, results in 2017
SGLT-2 inhibitors	DPP-1 inhibitors	100	6 months	Effects of treatment on the nominal change in arterial stiffness from baseline	Recruiting, results in 2017 (cardiovascular effects)
	Glimepiride				
Dapagliflozin (DEFINE-HF)	Placebo	250	12 weeks	Difference in the average reduction of NTproBNP	Not yet recruiting, results in 2017
Dapagliflozin (REFORM)	Placebo	58	Safety/efficacy study	Change in LV systolic- or diastolic volume	Recruiting, results in 2017
Dapagliflozin	Placebo	440	52 weeks	Mean change in HbA1c from baseline	Recruiting, results in 2019
	Saxagliptin				
	Glimpiride				

Continued



**Table 2** Continued

Compound	Comparator	n	Duration	Primary endpoint	References
Empagliflozin (JARDIANCE®)	Low dose–high dose	6000	156 weeks	Incidence of adverse drug reactions	Recruiting, results in 2020
T1DM					
Empagliflozin	Placebo	42	8 weeks	Change in GFR	75
Empagliflozin (EASE-3)	Placebo	960	52 weeks	Mean change in HbA1c from baseline	Recruiting, results in 2017
Empagliflozin (EASE-2)	Placebo	720	52 weeks	Mean change in HbA1c from baseline	Recruiting, results in 2017
Dapagliflozin	Placebo	768	52 weeks	Mean change in HbA1c from baseline	Recruiting, results in 2017
	Low dose–high dose				
	Insulin				

n, number of patients; T2DM, type 2 diabetes mellitus; T1DM, type 1 diabetes mellitus; HF, heart failure; LV, left ventricular.

body weight was related to a reduction in (epididymal) fat tissue.<sup>44,82</sup>

Clinical data have revealed a reduction of 2.2–3.3 kg body weight after 18–24 weeks of dapagliflozin treatment versus –0.9 to 1.55 kg weight gain in placebo-treated groups, depending on the initial body weight.<sup>59,60,62,63,80,81</sup> This drop in body weight was sustained but did not decrease further during a follow-up period of 52 weeks.<sup>61</sup> Empagliflozin, ipragliflozin, and canagliflozin showed a comparable dose-dependent weight reduction, combined with a reduction in waist circumference.<sup>71,73,84,86,87,89</sup> Combination therapy seemed to be beneficial in terms of the amount of weight loss.<sup>89</sup> Body weight reduction was also achieved on top of (inadequately controlled) insulin treatment, where the body weight gain due to insulin was not only inhibited, but in fact significantly reduced: 4.3–4.5 kg after 12 weeks.<sup>77,90</sup>

A study of body composition showed a numerically larger decrease in fat tissue mass compared with lean tissue mass in dapagliflozin-treated patients, in comparison to placebo-treated patients after 12 weeks, and even more after 24 weeks.<sup>107</sup> Additionally, it was observed that there was a shift from glucose oxidation to lipid oxidation in empagliflozin-treated patients,<sup>60</sup> indicating that the decrease in body weight appears to be associated with a loss of fat mass.

Others have investigated if intrusion of renal reabsorption would alter the uptake of electrolytes, which could consequently influence the bone mineral density, and eventually body weight.<sup>107</sup> Generally, no changes were observed in electrolytes concentrations, with the exception of small (and arguably physiologically irrelevant) increases in serum magnesium and phosphate.<sup>60,63,76</sup> Bone density was reduced in femoral and hip and increased in the spine, leading to no difference in bone turnover, and therefore no influence on overall body weight without an increased rate of bone fractures.<sup>107</sup>

Energy expenditure, measured by respiratory rate, heart rate, and body temperature, was unaffected by SGLT2 inhibition.<sup>61,92</sup> Long-term stagnation of body weight could therefore be explained by an increase in caloric intake.<sup>60</sup> This would correspond with pre-clinical data where nearly all animals showed an increase in chow consumption.

## Blood pressure

Hypertension is a very common co-morbidity of diabetes.<sup>108</sup> It may arise in diabetic patients due to sodium retention, to

hyperinsulinaemia and insulin resistance, and to baroreceptor impairment leading to sympathetic overdrive.<sup>109–111</sup> Hypertension is one of the main drivers of micro- and macrovascular complications by promoting endothelial dysfunction and decreased nitric oxide production.<sup>108,109</sup> Therefore, blood pressure reduction is an important element of vascular protection in diabetes mellitus.<sup>78</sup>

SGLT2 inhibition may lead to a decrease in blood pressure by the diuretic effect of glycosuria and increased sodium excretion through the inhibition of Na<sup>+</sup> reabsorption, which is coupled to glucose (Figure 2).<sup>2,76,88</sup> One study done by Osorio *et al.* showed that SGLT inhibition with phlorizin prevented the development of hypertension in rats with STZ-induced diabetes fed a normal diet or a high-salt diet.<sup>40</sup> This may indicate a role of SGLT in the development of hypertension, by lowering excessive sodium and water reabsorption.

The combination of SGLT2 inhibition with metformin led to an average decrease in SBP of ~4 mmHg, while diastolic blood pressure decreased by ~0–1.5 mmHg.<sup>59,61,67,71,73,84,112</sup> Combination therapy of a SGLT2 inhibitor with insulin effectively decreases blood pressure to a greater extent compared with metformin, while monotherapy with SGLT2 inhibition lowered blood pressure to a smaller extent.<sup>60,63,67,77,85</sup>

By looking at diastolic blood pressure, heart rate, and systolic blood pressure in several clinical trials, Chilton *et al.* calculated the index for ambulatory stiffness, a strong indicator for macrovascular disease. They found a favourable effect of empagliflozin on markers for arterial stiffness.<sup>113</sup> Additionally, empagliflozin treatment has been shown to decrease arterial stiffness and may also prevent the development of endothelial dysfunction in T1DM.<sup>75,114</sup>

A meta-analysis showed a decrease in systolic blood pressure, ranging from 3.6 to 6.6 mmHg.<sup>78–80</sup> This effect remained constant after a follow-up of 52–72 weeks.<sup>67,71,89</sup> These results indicate that SGLT2 inhibition might confer sustained blood pressure lowering effects. Of note, there was no increase in safety issues such as hypotension or dehydration found in these studies.<sup>86,89,95,115</sup>

## Uric acid

Serum uric acid levels have consistently been linked to CVD.<sup>116,117</sup> Excessive amounts of uric acid [over 6–7 mg/dL (360–416 µmol/L)] may alter endothelial function and stimulate smooth muscle cell

proliferation and the release of pro-inflammatory cytokines.<sup>117,118</sup> Serum uric acid is also strongly related to diabetic nephropathy in T1DM and T2DM patients. A decrease in serum uric acid has been described in response to SGLT2 inhibition.<sup>74,76,86</sup> These declines were apparent when SGLT2 inhibitors were tested both when applied as mono- and as add-on therapy, ranging from a decrease of 21–51  $\mu\text{mol/L}$ , independent of the drug and treatment time.<sup>59–63,67</sup> In contrast, several other oral antidiabetic treatments were related to an increase in uric acid of 16–20  $\mu\text{mol/L}$ .<sup>61,63</sup> SGLT2 inhibition may affect the level of serum uric acid due to the osmotic gradient of glycosuria. Uric acid is secreted from the tubular cells by glucose activation of the glucose–uric acid transporter (GLUT9b). An increase in glucose might lead to an increase in uric acid excretion and thus a decrease in serum uric acid.<sup>118</sup> Collectively, the effects of SGLT2 inhibition on uric acid might be beneficial in T2DM patients, while the effects in T1DM patients remain unknown.

## Haematocrit

Haematocrit, a measure for haemoglobin, has been related to CVD.<sup>119</sup> High haematocrit values are related to increases in blood viscosity, cardiac work, left ventricular hypertrophy, and thrombosis.<sup>79,119–121</sup> The risk for all-cause mortality and cardiac and infectious mortality rises with increasing percentages of haematocrit in diabetic patients.<sup>119</sup>

In response to SGLT2 inhibition with dapagliflozin or empagliflozin, haematocrit would increase by 1–3.5%, and with canagliflozin by 5.7–6.4%.<sup>59,61–63,67,71,84</sup> Placebo treatment led to a 1–2% decrease in haematocrit.<sup>59,67,71</sup> Increased values of haematocrit are most probably explained by the volume depletion of SGLT2 inhibition.<sup>60</sup> It is unclear whether this change is clinically relevant. Of note, there was no association found between SGLT2 inhibition and thrombosis.<sup>79</sup>

## Effect of sodium–glucose cotransporter 2 inhibition on the kidney: nephropathy and kidney disease

Because of the substantial effects of SGLT2 inhibition on the kidney, renal markers of diabetic nephropathy and kidney disease are separately discussed. Renal function is also highly predictable for severity of atherosclerotic disease.<sup>122</sup> Diabetic nephropathy is caused by glomerular and tubular injury, exemplified by hypertrophy of the basement membrane, tubules, epithelial mesenchymal transition, glycogen accumulation, and interstitial inflammation.<sup>118</sup> This is accompanied by (micro) albuminuria and a decline in GFR. By definition, nephropathy is characterized by a GFR of  $<60 \text{ mL/min}$  and/or by albuminuria of  $>300 \text{ mg/24 h}$ .<sup>61,122</sup> It has been suggested that SGLT2 inhibition may preserve renal function in both T1DM patients and T2DM patients. However, potential nephrotoxicity due to increased glucose exposure needs to be investigated.<sup>2</sup>

Generally, diabetic patients exhibit an increased GFR during early disease, so called hyperfiltration. Theoretically, this could be reduced by SGLT2 inhibition, due to a decreased reabsorption of sodium and glucose, and thereby initiating the tubuloglomerular feedback mechanism to reduce the filtration rate.<sup>24,106</sup> Pre-clinical

studies have showed that SGLT2 inhibition prevented the diabetic growth of the kidney and decreased glomerular size, albuminuria, and inflammation.<sup>37,55,115</sup> Additionally, gene expression of inflammatory markers was decreased in the glomerulus and cortex, macrophage infiltration was decreased, and oxidative stress in the tubular cells was significantly down-regulated.<sup>95</sup>

Clinical data of SGLT2 inhibition have shown a small decrease in GFR of  $\sim 0.5 \text{ mL/min/1.73 m}^{22}$  after 18 weeks of dapagliflozin treatment and a decrease of 2–3.8  $\text{mL/min/1.73 m}^{22}$  after 104 weeks of canagliflozin treatment. However, this was in both cases more pronounced in the group receiving a reference drug (decrease of 5–6  $\text{mL/min/1.73 m}^{22}$  with glimepiride or glipizide, respectively).<sup>61,87</sup> Other studies showed no difference in GFR.<sup>62,71,84</sup> A meta-analysis concluded that SGLT2 inhibition has no major effect on GFR,<sup>76</sup> or a small decrease in GFR without a change in albuminuria.<sup>79</sup> An explanation could be that clinical trials usually select for patients with relatively preserved renal function at the commencement of the study, and that the duration of these studies is not long enough to properly study the (inhibition of the) development of renal disease. The most recent trial with a follow-up of 3 years showed a lower incidence of patients with acute renal failure in empagliflozin-treated groups compared with placebo groups,<sup>74</sup> which may hint towards a protective effect of SGLT2 inhibition on renal function.

## Sodium–glucose cotransporter 2 inhibition in the diabetic heart

Even though there is a negligible expression of the SGLT2 transporter in the myocardium,<sup>123,124</sup> inhibitors may have an influence on the cardiac tissue via other mechanisms.

Collagen accumulation and sensitivity to catecholamines may be altered in the diabetic heart, as well as destructive neuropathy affecting sympathetic and parasympathetic nerve endings leading to altered electromechanical conduction. Indeed, patients with diabetes are more susceptible to spontaneous arrhythmias and third-degree AV block.<sup>125,126</sup> Myocytes isolated from STZ-induced diabetic rats have amplitude shortening and reduction of the calcium transient amplitude in response to dapagliflozin in comparison with myocytes from control rats, indicating a decrease in myocyte contraction force in response to SGLT2 inhibitor. However, this presumably negative inotropic effect of dapagliflozin has been measured only after short-term exposure, indicating the development of tolerance to the effects of dapagliflozin.<sup>127</sup> There was no effect of dapagliflozin found on QT/QT intervals of healthy patients (a measure to evaluate ventricular repolarization of a drug).<sup>128</sup>

A recent study investigated the influence of empagliflozin on the diabetic heart by looking at fibrosis, coronary arterial thickening, inflammation, and oxidative stress in the hearts of diabetic obese *db/db* mice. The authors showed that 10 weeks of treatment significantly reduced cardiac interstitial fibrosis, perivascular fibrosis, coronary arterial thickening, cardiac interstitial macrophage infiltration, and cardiac superoxide levels. Furthermore, endothelial function improved, indicated by an enhanced vasodilatory response to acetylcholine.<sup>129</sup> These results may indicate a cardioprotective function of SGLT2 inhibition in diabetic patients. Thus far, the effects of SGLT2 inhibition on neuropathy or retinopathy have not been established.



## Sodium–glucose cotransporter 2 inhibition and cardiovascular outcome

The effects of SGLT2 inhibition on CV outcome may be assessed by investigating diabetic complications, both in the small vessels and in larger vessels translating into CV events. Vascular dysfunction and endothelial damage in the smaller vessels are often caused by hyperglycaemia due to non-enzymatic glycation, which leads to oxidative stress, which can be measured by an increase in reactive oxygen species.<sup>6,109,130,131</sup> Macrovascular complications such as myocardial infarction, stroke, and peripheral vascular disease are usually caused by the acceleration of atherosclerosis.<sup>100,109,132</sup> It has been shown that intensive glycaemic control with sulfonylurea derivatives, insulin, dietary restriction, or metformin leads to a 24% decrease in microvascular disease and a 33% decreased risk of myocardial infarction after 10 years.<sup>1,4,133</sup>

How does SGLT2 inhibition compare?

## Sodium–glucose cotransporter 2 inhibition and prevention of major cardiovascular events

Most clinical studies testing SGLT2 inhibition reported no or a low incidence of serious adverse events, which could be due to the size and duration of these trials,<sup>62,63,71,92,93</sup> and the number was numerically equal to control patients.<sup>86,87</sup> Meta-analyses suggested benefit of SGLT2 inhibition on CV function compared with placebo, although this was found to be equal when compared with other anti-diabetic therapies.<sup>78,79</sup> However, a recent randomized, placebo-controlled trial of Zinman *et al.*, the EMPA-REG study, prospectively evaluated if SGLT2 inhibition could reduce hard CV endpoints.<sup>74</sup> They tested the value of empagliflozin in 7020 patients with T2DM, and followed long term (median follow-up of 3.1 years). Primary outcome was a composite of CV mortality, non-fatal myocardial infarction (MI), and non-fatal stroke. Treatment with empagliflozin resulted in a decreased risk for the primary outcome compared with placebo patients [hazard ratio (HR) 0.85; 95% CI 0.74–0.99;  $P = 0.04$  for superiority]. There were no clear differences in non-fatal MI and non-fatal stroke. But empagliflozin significantly reduced the CV mortality (38% relative risk reduction) and all-cause mortality (32% relative risk reduction). Interestingly, hospitalization due to heart failure was also reduced. In a sub-study of EMPA-REG, Fitchett *et al.* specifically investigated the impact of empagliflozin treatment on heart failure. Their results showed a substantial decrease in heart failure (HR 0.65; 95% CI 0.50–0.85) and CV mortality (HR 0.65; 95% CI 0.49–0.77) in T2DM patients with and without baseline heart failure.<sup>74,134</sup> Although up until now we have only one large RCT, the results of EMPA-REG were welcomed and have prompted several more studies that will test the hypothesis that SGLT2 inhibitors may effectively reduce hard CV endpoints.

The question remains whether the beneficial effects of SGLT2 inhibition on outcome is related to the strong diuretic effect or through other mechanisms of action, and future studies should provide more mechanistic detail. Although these results need to be validated, the current studies provide strong suggestion that

SGLT2 inhibitors may reduce the incidence of CV events and also, in particular, new onset heart failure.

## Safety

So far, SGLT2 inhibition and glycosuria appear well tolerated and have not been associated with excessive side effects with a low chance at developing hypoglycaemia.<sup>79,80,83,135</sup> But few concerns exist, such as a potentially deleterious effects on kidney function or excessive water loss and dehydration.<sup>21,39</sup> Glucose excretion via urine increases the risk of genitourinary infections, usually presented as genital infections (~4% increase compared with placebo) and sometimes shown in urinary tract infections (~1% increase compared with placebo).<sup>53</sup> These infections were generally considered non-severe and could easily be treated with antibiotic treatment.<sup>12,39,53</sup> A potentially disturbing tendency towards an increased incidence of breast and bladder cancer has been reported—clearly this needs to be investigated more intensively in clinical trials.<sup>2,53</sup> Reassuringly, animal studies with doses up to 100 times the clinical dosages did not show carcinogenic mutagenesis.<sup>22</sup>

Concerns might be the binding to SGLT1 or other locations of SGLT2 expression, and its indirect effects on vital tissues, such as brain. In addition, studies have shown that glucose absorption might be delayed during SGLT2 inhibition.<sup>85</sup> More long-term studies will be required to reveal other possible side effects.

## Conclusion

SGLT2 inhibition is the most recent addendum to the arsenal of oral antidiabetic agents. Data thus far suggest it to be a safe and tolerable intervention, which reduces hyperglycaemia by urinary glucose excretion. SGLT2 inhibition can be prescribed as a monotherapy, but is easily combined with other interventions, where it further reduces target values of inadequately controlled patients. The benefits of this class of drugs may be that they function in an insulin-independent manner by which they reduce HbA1c values and plasma glucose and improve insulin sensitivity and the functioning of  $\beta$ -cells. Furthermore, it has a favourable CV profile regarding decreases in blood pressure and body weight, which are maintained over a prolonged period of time.<sup>74</sup> A reduction in uric acid may reduce the risk of developing atherosclerosis and associated diseases, as well as the small shift in the lipid balance. Pre-clinical data have suggested that the cardioprotective effect of SGLT2 inhibition may be conferred by the reduction of ROS and cardiac interstitial fibrosis.<sup>129</sup> A large trial indeed suggested that all-cause mortality and new onset heart failure were reduced in patients using SGLT2 inhibitors,<sup>74</sup> and that diabetic patients at high CV risk were better protected against heart failure with SGLT2 inhibition compared with placebo groups.<sup>134</sup> The most common side effect is genitourinary infection, which is however easily treated.

Several additional studies are needed before we can recommend SGLT2 inhibition for a wide array of patients. Currently, many studies are recruiting to investigate the effect of SGLT2 inhibition in patients with T1DM (Table 2). Second, another area of uncertainty is if SGLT2 inhibition would be suitable for patients with a compromised glomerular filtration<sup>12,18,53</sup> and what the impact of the high exposure to sodium and glucose in the kidney could be in the long term.<sup>88</sup>

However, no major effects on albumin secretion or GFR have been observed, and possible protective effects have been reported.<sup>74</sup> Third, with respect to its presumed organ protective effects, it would be interesting to know if SGLT2 inhibition could be a beneficial add-on therapy in diabetic patients who have to recover from earlier CV events. Given the very promising and encouraging results thus far, SGLT2 inhibition has the potency to become the drug of choice for patients suffering from diabetes with a high risk at heart disease.

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